

REMARKS

Objections

Claim 3 is objected to because the word "Venezuelan" is misspelled in the claim.
Claim 7 is objected to because of improper Markush claiming and the duplicate commas after the term "PfCSP."

Response

The typographic error in claim 3 is corrected in this amendment. Claim 7 is canceled and its limitations are incorporated into claim 1. Therefore, applicants respectfully request the objections be withdrawn.

Claim Rejections – 35 USC §112

Claim 7 is drawn to a method to immunize a subject against malarial disease comprising: administering to the subject a priming immunization preparation comprising one or more alphavirus replicons expressing a gene encoding a malarial antigen or combination of malarial antigens; and subsequently administering to the subject a boosting immunization preparation comprising the malarial antigen or combination of malarial antigens, wherein said malarial antigen is selected from the group consisting of: PfCSP, PfEXP1, PfSSP2, PfLSA-1, PfLSA-3, PfMSP-1, PfAMA-1, PfEBA-175, PfMSP-3, PfMSP-4, PfMSP-5, PfRAP-1 and PfRAP-2.

Claim 7 is rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The examiner argues that "PfCSP, PfEXP1, PfSSP2, PfLSA-1, PfLSA-3, PfMSP-1, PfAMA-1, PfEBA-175, PfMSP-3, PfMSP-4, PfMSP-5, PfRAP-1,

PfRAP-2” constitute laboratory designation engendering no specific sequence.

Moreover, the examiner contends said terms are referring to antigens wherein they are purportedly genes. Consequently, it is impossible to determine the metes and bounds of the claimed invention.

Response

Applicants contend that “PfCSP, PfEXP1, PfSSP2, PflSA-1, PflSA-3, PfMSP-1, PfAMA-1, PfEBA-175, PfMSP-3, PfMSP-4, PfMSP-5, PfRAP-1, and PfRAP-2” are not laboratory designations. They are terms routinely used in this area of research designating specific malarial antigens or proteins, and are stated as such in many publications. For example, Wang et al. studied the CD8(+) T- cell response to DNA encoding multiple *P. falciparum* proteins, such as PfCSP, PfSSP2, PfExp-1 and PflSA-1. (R. Wang et al., Simultaneous induction of multiple antigen-specific cytotoxic T lymphocytes in nonhuman primates by immunization with a mixture of four *Plasmodium falciparum* DNA plasmids; Infect. Immun., 1998 Set; 66(9):4193-202.) Similar usage of these terms can be found in the abstracts submitted in support of this paper.

Accordingly, applicants contend that the mete and bounds of the invention is clearly defined and distinguish claimed in claim 7. A person having ordinary skill in the art would be able to identify the specific sequences based on this disclosure. Thus, applicants respectfully request that the rejection under 35 USC §112, second paragraph be reconsidered and withdrawn.

Claim Rejections – 35 USC 103

Claims 1-17 are rejected under 35 USC §103(a) as being unpatentable over McMichael et al. (WO 98/56919 – IDS filed on 4-11-08) and Sallberg et al. (US patent application publication US 2002/0165172).

The newly amended claim 1 is drawn to a method to immunize a subject against malarial disease comprising: a) administering to the subject a priming immunization preparation comprising one or more alphavirus replicon expressing a gene encoding a malarial antigen or combination of malarial antigens; and b) subsequently administering to the subject a boosting immunization preparation comprising the malarial antigen or combination of malarial antigens, wherein said preparation is a recombinant non-alphavirus viral expression system encoding the malarial antigen; wherein the malarial antigen is selected from the group consisting of: PfCSP, PfEXP1, PfSSP2, PfLSA-1, PfLSA-3, PfMSP-1, PfAMA-1, PfEBA-175, PfMSP-3, PfMSP-4, PfMSP-5, PfRAP-1, and PfRAP-2.

Claim 17 is directed to a method to immunize against malarial disease, comprising priming with a VEE replicon particles expressing a gene encoding a malarial antigen or immunogenic fragment thereof, and boosting with an immunization preparation comprising poxvirus encoding the malarial antigen, wherein the malarial antigen is selected from the group consisting of: PfCSP, PfEXP1, PfSSP2, PfLSA-1, PfLSA-3, PfMSP-1, PfAMA-1, PfEBA-175, PfMSP-3, PfMSP-4, PfMSP-5, PfRAP-1, and PfRAP-2.

Response

McMichael et al. disclose methods of inducing CD8 T cell immune response to malarial antigens comprising the administration of a priming composition of a nucleic acid (DNA or RNA), which may be either packaged or in free form; and a boosting composition comprises a non-replicating or replication-impaired pox virus vector, which may be a Ty-VLP or a recombinant adenovirus. McMichael et al. further disclose that MVA may be used in both priming or boosting composition and other viral vectors, such as herpes virus can be used in the priming composition. However, McMichael, as the examiner noted, do not explicitly teach the use of alphavirus generally, or the use of VEE virus specifically. McMichael et al also fail to teach the specific malarial antigens encoded by said alphavirus replicon and used in the boosting immunization preparation.

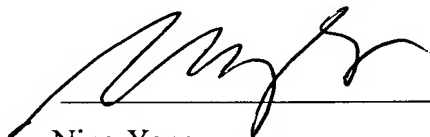
Sallberg et al disclose a method for treating intracellular infections within warm-blooded animals, comprising: (a) administering to a warm-blooded animal a vector construct, which directs the expression of at least one immunogenic portion of an antigen derived from an intracellular pathogen; and (b) administering to said warm-blooded animal a protein which comprises said immunogenic portion of said antigen, such that an immune response is generated. Sallberg further discloses that the method may be used to treat malaria and said vector construct may be carried by an alphavirus. However, Sallberg does not teach the specific use of VEE in the priming immunization. Sallberg also fail to teach the specific malarial antigens encoded in the priming preparation and used in boosting preparation except PfCSP.

As such, McMichael in view of Sallberg, fail to teach the use of VEE in the priming immunization and therefore claim 17. Applicants respectfully request the rejection against claim 17 be reconsidered and withdrawn.

In addition, the combined prior art references do not teach the specific malarial antigens or combination of antigens disclosed in this application, such as those recited in claim 7. Because these antigens are critical components of the immunization preparation of the present invention, and are responsible for the preparation's immunogenicity against malaria, an immunization method without clearly defined malarial antigens may not initiate any immune response against malaria. Therefore, McMichael and Sallberg combined do not teach the invention recited by the amended claim 1, which incorporated the limitations of claim 7. Claim 6 and 7 are hereby canceled. Applicants respectfully request the rejections against newly amended claim 1 and its dependent claims be reconsidered and withdrawn.

The commissioner is authorized to deduct any fees and credit any overpayments using USPTO deposit account No. 140,595

Respectfully submitted,



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cc: Kimpel Janice, AlphaVax Inc.

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1: Infect Immun. 1998 Sep;66(9):4193-202.

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Simultaneous induction of multiple antigen-specific cytotoxic T lymphocytes in nonhuman primates by immunization with a mixture of four Plasmodium falciparum DNA plasmids.**Wang R, Doolan DL, Charoenvit Y, Hedstrom RC, Gardner MJ, Hobart P, Tine J, Sedegah M, Fallarme V, Sacchi JB Jr, Kaur M, Klinman DM, Hoffman SL, Weiss WR.**

Malaria Program, Naval Medical Research Institute, Bethesda, Maryland 20889, USA.

CD8(+) T cells have been implicated as critical effector cells in protective immunity against malaria parasites developing within hepatocytes. A vaccine that protects against malaria by inducing CD8(+) T cells will probably have to include multiple epitopes on the same protein or different proteins, because of parasite polymorphism and genetic restriction of T-cell responses. To determine if CD8(+) T-cell responses against multiple *P. falciparum* proteins can be induced in primates by immunization with plasmid DNA, rhesus monkeys were immunized intramuscularly with a mixture of DNA plasmids encoding four *P. falciparum* proteins or with individual plasmids. All six monkeys immunized with PfCSP DNA, seven of nine immunized with PfSSP2 DNA, and five of six immunized with PfExp-1 or PfLSA-1 DNA had detectable antigen-specific cytotoxic T lymphocytes (CTL) after in vitro restimulation of peripheral blood mononuclear cells. CTL activity was genetically restricted and dependent on CD8(+) T cells. By providing the first evidence for primates that immunization with a mixture of DNA plasmids induces CD8(+) T-cell responses against all the components of the mixture, these studies provide the foundation for multigene immunization of humans.

PMID: 9712767 [PubMed - indexed for MEDLINE]

PMCID: PMC108505

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Induction in humans of CD8+ and CD4+ T cell and antibody responses by sequential immunization with malaria DNA and recombinant protein. [J Immunol. 2004]

Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the Plasmodium falciparum sporozoite surface protein 2. [J Exp Med. 1995]

A DNA vaccine encoding the 42 kDa C-terminus of merozoite surface protein 1 of Plasmodium falciparum induces antibody, interferon-gamma and cytotoxic T cell responses in rhesus monkeys: immuno-stimulatory effects of granulocyte macrophage-colony stimulating factor. [Immunol Lett. 2002]

Review The development of a multivalent DNA vaccine for malaria. [Springer Semin Immunopathol. 1997]

Review T cell responses to repeat and non-repeat regions of the circumsporozoite protein detected in volunteers immunized with Plasmodium falciparum sporozoites. [J Immunol. 1992]

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1: Infect Immun. 1997 Aug;65(8):3430-7.

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Development of two monoclonal antibodies against Plasmodium falciparum sporozoite surface protein 2 and mapping of B-cell epitopes.**Charoenvit Y, Fallarme V, Rogers WO, Sacchi JB Jr, Kaur M, Aguiar JC, Yuan LF, Corradin G, Andersen E, Wizel B, Houghten RA, Oloo A, De la Vega P, Hoffman SL.**Malaria Program, Naval Medical Research Institute, Bethesda, Maryland 20889-5607, USA.
charoenvit@nmripo.nmri.nmci.navy.mil

The Plasmodium yoelii sporozoite surface protein 2 (PySSP2) is the target of protective cellular immunity. Cytotoxic T cells specific for the Plasmodium falciparum analog PfSSP2, also known as thrombospondin-related anonymous protein (TRAP), are induced in human volunteers immunized with irradiated sporozoites. PfSSP2 is an important candidate antigen for a multicomponent malaria vaccine. We generated and characterized three monoclonal antibodies (MAbs) specific for PfSSP2/TRAP. The MAbs PfSSP2.1 (immunoglobulin G1 [IgG1]), PfSSP2.2 (IgG2a), and PfSSP2.3 (IgM) were species specific and identified three distinct B-cell epitopes containing sequences DRYI, CHPSDGKC, and TRPHGR, respectively. PfSSP2.1 partially inhibited P. falciparum liver-stage parasite development in human hepatocyte cultures (42 and 86% in two experiments at 100 microg/ml). Mice immunized with vaccinia virus expressing full-length PfSSP2 protein produced antibodies to (DRYIPYSP)3, and humans living in malaria-endemic areas (Indonesia and Kenya), who have lifelong exposure and partial clinical immunity to malaria, had antibodies to both (DRYIPYSP)3 and (CHPSDGKCN)2. Mice immunized with multiple antigen peptides MAP4 (DRYIPYSP)3P2P30 and MAP4 (CHPSDGKCN)3P2P30 in TiterMax developed antibodies to sporozoites that partially inhibited sporozoite invasion of human hepatoma cells (39 to 71% at a serum dilution of 1:50 in three different experiments). The modest inhibitory activities of the MAbs and the polyclonal antibodies to PfSSP2/TRAP epitopes do not suggest that a single-component vaccine designed to induce antibodies against PfSSP2/TRAP will be protective. Nonetheless, the MAbs directed against PfSSP2, and the peptides recognized by these MAbs, will be essential reagents in the development of PfSSP2/TRAP as a component of a multivalent P. falciparum human malaria vaccine.

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Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the Plasmodium falciparum sporozoite surface protein 2. [Exp Med. 1995]

HLA-A2-restricted cytotoxic T lymphocyte responses to multiple Plasmodium falciparum sporozoite surface protein 2 epitopes in sporozoite-immunized volunteers [Immunol. 1995]

Induction of murine cytotoxic T lymphocytes against Plasmodium falciparum sporozoite surface protein 2. [Exp Med. 1994]

Review T cell responses to repeat and non-repeat regions of the circumsporozoite protein detected in volunteers immunized with Plasmodium falciparum sporozoites. [Immunol. 1992]

Review Pre-erythrocytic malaria vaccine: mechanisms of protective immunity and human vaccine development. [Parasitol. 1999]

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1: J Eukaryot Microbiol. 1998 Jan-Feb;45(1):131-6.

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Stable patterns of allelic diversity at the Merozoite surface protein-1 locus of *Plasmodium falciparum* in clinical isolates from southern Vietnam.

Ferreira MU, Liu Q, Zhou M, Kimura M, Kaneko O, Van Thien H, Isomura S, Tanabe K, Kawamoto F.

Department of Medical Zoology, Nagoya University School of Medicine, Japan. muferrei@usp.br

The extent of allelic diversity at the Merozoite Surface Protein-1 locus of *Plasmodium falciparum* (PfMSP-1) was examined in isolates collected from symptomatic patients living in a mesoendemic area in southern Vietnam. The variable blocks 2, 4 and 10 were typed by polymerase chain reaction and 24 PfMSP-1 gene types were defined as unique combinations of allelic types detected in each variable block. Nineteen PfMSP-1 gene types were identified and 182 parasite populations were fully typed among 102 isolates. Forty-eight (47%) patients harbored more than one typed parasite population, and one patient had at least eight genetically distinct subpopulations. As previously shown in the same endemic area, recombination between blocks 4 and 10 was significantly less frequent than expected from random assortment of allelic types. The distribution of PfMSP-1 gene types, however, did not differ significantly from that observed in isolates collected in the same area 17-24 mo before the present study. Furthermore, the prevalence of the most common gene types and the average number of different gene types harbored by the same host did not decrease with age. This argues against the prominence of frequency-dependent immune selection of PfMSP-1 polymorphisms in this parasite population.

PMID: 9495041 [PubMed - indexed for MEDLINE]

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Allelic diversity at the merozoite surface protein-1 locus of *Plasmodium falciparum* in clinical isolates from the southwestern Brazilian Amazon. [Am J Trop Med Hyg. 1998]

Allelic diversity at the merozoite surface protein-1 (MSP-1) locus in natural *Plasmodium falciparum* populations: a brief overview. [Mem Inst Oswaldo Cruz. 1998]

Geographical patterns of allelic diversity in the *Plasmodium falciparum* malaria-vaccine candidate, merozoite surface protein-2. [Ann Trop Med Parasitol. 2001]

Review Markers for population genetic analysis of human plasmodia species, *P. falciparum* and *P. vivax*. [Emerg Dis. 2003]

Review A PCR method for molecular epidemiology of *Plasmodium falciparum* Msp-1. [Tokai J Exp Clin Med. 1998]

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1: J Biol Chem. 2001 Aug 17;276(33):31311-20. Epub 2001 Jun 8.

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J Biol Chem**Proteolytic processing and primary structure of Plasmodium falciparum apical membrane antigen-1.****Howell SA, Withers-Martinez C, Kocken CH, Thomas AW, Blackman MJ.**

Division of Protein Structure and the Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom.

Plasmodium falciparum apical membrane antigen-1 (PfAMA-1) is a malaria merozoite integral membrane protein that plays an essential but poorly understood role in invasion of host erythrocytes. The PfAMA-1 ectodomain comprises three disulfide-constrained domains, the first of which (domain I) is preceded by an N-terminal prosequence. PfAMA-1 is initially routed to secretory organelles at the apical end of the merozoite, where the 83-kDa precursor (PfAMA-1(83)) is converted to a 66-kDa form (PfAMA-1(66)). At about the time of erythrocyte invasion, PfAMA-1(66) selectively translocates onto the merozoite surface. Here we use direct microsequencing and mass spectrometric peptide mass fingerprinting to characterize in detail the primary structure and proteolytic processing of PfAMA-1. We have determined the site at which processing takes place to convert PfAMA-1(83) to PfAMA-1(66) and have shown that both species possess a completely intact and unmodified transmembrane and cytoplasmic domain. Following relocation to the merozoite surface, PfAMA-1(66) is further proteolytically cleaved at one of two alternative sites, either between domains II and III, or at a membrane-proximal site following domain III. As a result, the bulk of the ectodomain is shed from the parasite surface in the form of two soluble fragments of 44 and 48 kDa. PfAMA-1 is not detectably modified by the addition of N-linked oligosaccharides.

PMID: 11399764 [PubMed - indexed for MEDLINE]

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A single malaria merozoite serine protease mediates shedding of multiple surface proteins by juxtamembrane cleavage. [J Biol Chem. 2003]

Invasion-inhibitory antibodies inhibit proteolytic processing of apical membrane antigen 1 of Plasmodium falciparum merozoites. [Proc Natl Acad Sci U S A. 2003]

Structure and inter-domain interactions of domain II from the blood-stage malarial protein, apical membrane antigen-1. [Mol Biol Cell. 2005]

Review Proteins on the surface of the malaria parasite and cell invasion. [Parasitology. 1994]

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☐ 1: [Parasite Immunol.](#) 2008 Oct;30(10):497-514. Epub 2008 Jun 28.

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Blood stage malaria antigens induce different activation-induced cell death programs in splenic CD4+ T cells.

Mukherjee P, Devi YS, Chauhan VS.

International Centre of Genetic Engineering and Biotechnology, Aruna Asaf Ali Road, New Delhi, India. paushali@icgeb.res.in

CD4(+) T cells respond to antigen immunization through a process of activation, clonal expansion to generate activated effector T cells followed by activation-induced clonal deletion of the responding T cells. While loss of responding T cells in post-activation death by apoptosis is a major factor regulating immune homeostasis, the precise pathways involved in downsizing of *Plasmodium falciparum* antigen-induced T cell expansions are not well characterized. We report in this study that splenic CD4(+) T cells from mice immunized with nonreplicating immunogens like OVA or recombinant blood stage *P. falciparum* antigens, PfMSP-3 and PfMSP-1(19) or crude parasite antigen (PfAg) undergo sequential T cell activation, proliferation followed by activation-induced cell death (AICD) in a dose- and time-dependent manner after Ag restimulation. While PfMSP-3 and OVA-induced AICD was mediated through a death receptor-dependent apoptotic program, PfMSP-1(19) and PfAg-induced AICD was via a mechanism dependent on the activation of mitochondria apoptosis signalling pathway through Bax activation. These results provide insights into the mechanism through which two blood stage merozoite antigens trigger different apoptotic programs of AICD in splenic CD4(+) T cells.

PMID: 18643960 [PubMed - indexed for MEDLINE]

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T-cell recognition of a cross-reactive antigen(s) in erythrocyte stages of *Plasmodium falciparum* and *Plasmodium yoelii*: inhibition of parasitemia by this antigen(s). [Infect Immun. 1993]

Primed T cells are more resistant to Fas-mediated activation-induced cell death than naive T cells. [J Immunol. 1999]

Complete protective immunity induced in mice by immunization with the 19-kilodalton carboxyl-terminal fragment of the merozoite surface protein-1 (MSP1[19]) of *Plasmodium yoelii* expressed in *Saccharomyces cerevisiae*: correlation of protection with antigen-specific antibody titer, but not with effector CD4+ T cells. [J Immunol. 1997]

Review T cell responses to repeat and non-repeat regions of the circumsporozoite protein detected in volunteers immunized with *Plasmodium falciparum* sporozoites. [J Immunol. 1992]

Review Antigen-induced T cell death is regulated by CD4 expression. [Int Rev Immunol. 2001]

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Evaluation of three *Pichia pastoris*-expressed *Plasmodium falciparum* merozoite proteins as a combination vaccine against infection with blood-stage parasites.

Zhang D, Pan W.

Department of Etiologic Biology, Second Military Medical University, Shanghai, China.

Because invasion of erythrocytes by *Plasmodium falciparum* merozoites involves multiple receptor-ligand interactions, it may be necessary to develop a multivalent malaria vaccine that is comprised of distinct parasite ligands. PfAMA-1, PfMSP1, and PfEBA-175 are merozoite proteins that play important roles in invasion. We have constructed a PfCP-2.9 chimeric protein consisting of PfAMA-1 and PfMSP1 and tested it for immunogenicity in animal models and humans. The F2 subdomain of PfEBA-175 (PfEBA-175II F2) was identified as the binding domain for glycophorin A on erythrocytes. In this study, we used the codon frequencies of the yeast *Pichia pastoris* to redesign and synthesize a gene encoding the F2 domain. We found that the codon-optimized gene was expressed at a high level in *P. pastoris* as a soluble protein with a yield of about 300 mg/liter. The expressed protein was able to bind normal erythrocytes but not those treated with neuraminidase or trypsin. Moreover, the protein was recognized by the sera of malaria patients and was highly immunogenic in mice, rabbits, and rhesus monkeys. Immunoglobulin G isolated from both immunized rabbits and monkeys inhibited in vitro parasite growth. Immunization of animals with a combination of PfEBA-175II F2 and PfCP-2.9 did not result in antigen (Ag) competition in animals. Moreover, antibodies to both PfEBA-175II F2 and PfCP-2.9, isolated from rabbits immunized with both constructs, inhibited parasite growth in vitro. The combination of high yield, functional folding, antibody inhibition, and lack of Ag competition provides support for inclusion of these merozoite proteins in a combination vaccine against infection with blood-stage parasites.

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Effect of codon optimization on expression levels of a functionally folded malaria vaccine candidate in prokaryotic and eukaryotic expression systems. [Infect Immun. 2003]

Malaria vaccine-related benefits of a single protein comprising *Plasmodium falciparum* apical membrane antigen 1 domains I and II fused to a modified form of the 19-kilodalton C-terminal fragment of merozoite surface protein 1. [Infect Immun. 2007]Review Antibodies and *Plasmodium falciparum* merozoites. [Trends Parasitol. 2001]

Review The development of a multivalent DNA vaccine for malaria. [Springer Semin Immunopathol. 1997]

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